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Metastable State of a Protein Crystal

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Abstract

The dehydration-induced shrinkage and changes in mechanical properties of glutaraldehyde-treated tetragonal crystals of hen egg white lysozyme are shown to follow different paths depending upon the period of exposure of the sample to each intermediate level of humidity. The crystals were found to exist in a metastable state after a rapid decrease of the relative humidity to 60–70%.

Introduction

To elucidate the role of water in protein structure and function, and to study the effects of dehydration on protein properties a large number of experiments have been carried out with solid protein samples (amorphous films, crystals, freeze-dried samples). The protein properties may change in these experiments due not only to the dehydration of groups on the protein surface, but also to the deformation of the molecule which is subjected to some internal pressure arising from the removal of water from the channels between protein globules (Laplace's pressure). The protein molecule in a solid sample is subjected to a complex inhomogeneous deformation tending to change its shape in such a way as to reduce the size of the voids developed after the water removal. In the course of this deformation the molecules indent each other and come closer causing shrinkage of the sample. The deformation is known to be elastic in solids only until the stress reaches its ultimate value. On being subjected to the ultimate stress and/or loaded for a long time the solid may deform inelastically displaying discontinuities in the stress–strain curve, irreversibility and creep flow.

We present here some data suggesting a similar inelastic deformation to be inherent in drying the tetragonal crystals of lysozyme.

Materials and methods

Tetragonal crystals of hen egg white lysozyme were grown from an Olainensky product according to the procedure of Steinrauf (1959). Glutaraldehyde cross-linking of the crystals, microtome sectioning and sample preparation procedures, as well as the methods for measuring Young's modulus E and the logarithmic decrement ϑ were described earlier (Morozov & Morozova, 1981). The measurements were made at 298 K in a thermostatted humidity chamber furnished with an air agitator. The humidity in the chamber was kept constant by a drop of $CaCl_2$ or LiCl solution or by passing through the chamber a small flow of air dried over silica gel. The length of the sample was measured in the chamber by an eyepiece micrometer with an accuracy of about 0·3%.

In measuring the shrinkage of the crystals by X-ray diffraction we used a device similar to that described by Einstein & Low (1962). A crystal or a microtome section was mounted in a quartz capillary 10–15 mm away from the drop of $CaCl_2$. Neither air agitation and thermostatting nor evacuation (Huxley & Kendrew, 1953) was used. ($hk0$), ($h0l$) and ($0kl$) reciprocal-lattice sections with $\mu = 15°$ were taken for crystals which had been stored for 1–2 d at a given humidity. The same sections were taken with $\mu = 5°$ over a period of 2–3 d for the microtome sections 30 μm thick.

Results and discussion

The humidity-dependent shrinkage of the tetragonal lysozyme crystals in the [001] direction measured under different conditions and with different methods is shown in Fig. 1. The lattice cell dimensions of the crystals and microtome sections measured with the X-ray diffraction method and the length of the crystal plates measured with a light microscope are seen to display similar relative changes when the samples are dried in similar conditions. Such a similarity makes it possible to measure the crystal cell shrinkage with a rapid and convenient method under a light microscope. X-ray diffraction patterns of ($0kl$), ($h0l$) and ($hk0$) reciprocal-lattice sections of the crystals and microtome sections are identical at high humidity and change identically upon drying, ruling out any considerable changes of the crystal structure in the microtome-sectioning procedure.

On slow dehydration the decrease in the lattice dimension 'c' is seen from curve (1) of Fig. 1 to occur smoothly, the greatest changes being in the humidity range $A > 70\%$. The 'a' and 'b' parameters decrease in a similar manner, yet their relative decrease is about half that of 'c'. Along with the changes in the lattice

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dimensions a gradual fading out of the diffraction pattern and decrease in minimum X-ray spacing similar to that described for haemoglobin and insulin (Einstein & Low, 1962; Perutz, 1942) is observed. The minimum spacing of the crystals increases from $d_{min} \leq$ 2·5 Å in the mother liquid to 4 Å at $A = 80\%$. At $A = 75\%$ it reaches the maximum value $d_{min} = 6{\cdot}5$ Å and does not change on drying to the lowest humidity. The space group $P4_32_12$ remains unchanged on drying as far as can be deduced from the limited number of systematic absences at low resolution.

Curve (3) in Fig. 1 presents an essentially different behaviour of the crystals on rapid change of humidity. Such measurements are possible only with thin crystal plates (rapid dehydration) using the light microscope (rapid measurements of crystal deformation). The study of the adsorption isotherm for water uptake by the crystals has shown that in the humid chamber with air agitation the hydration of the crystals 0·2-0·5 mm in size reached its equilibrium level within 30-60 min and did not change upon subsequent daily storage at the same humidity (Gevorkian & Morozov, 1983). This suggests that equilibrium of thin crystal plates (5-10 mm thick) is completed within 1 min provided the rate of the equilibration is determined by water diffusion through the crystal. This assumption is supported by the behaviour of Young's modulus after the change in humidity in the chamber.

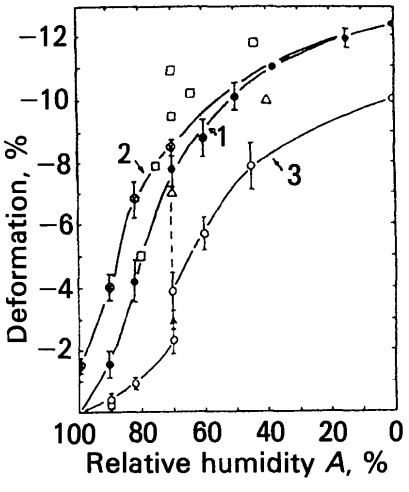

Fig. 1. The humidity dependence of the relative shrinkage of tetragonal $P4_32_12$ lysozyme crystals in the [001] direction measured as length changes of crystal plates after storage for 2-3 d at each humidity point on decreasing (●) and subsequently increasing (⊗) the humidity; (○) the same measured after 15-30 min exposure to the humidity; (△) and (□) the same measured by X-ray diffraction as the 'c' parameter changes on slow (2-3 d at each humidity) drying the microtome sections and crystals 0·2-0·4 mm in size respectively. The measurements were made on samples soaked in the buffer used for the crystal growth at 298 K. The step-like transition is denoted by arrows, and the subsequent relaxation process by a dashed line. The points with the r.m.s.d.'s shown are the means obtained in 10-25 experiments, others being the results of single measurements.

In spite of its strong humidity dependence, shown in Fig. 3, the modulus is seen in Fig. 2 to reach its equilibrium 1-3 min after the humidity change and to have no hysteresis on rehydration in the region $A > 70\%$.

Comparison of curves (3) and (1) in Fig. 1 shows that complete deformation of the crystal corresponding to the hydration level at a given humidity fails to occur on rapid change of the humidity and results in accumulation of some internal stresses. On reaching their limits these stresses are seen in Fig. 1 to cause a spontaneous step-like deformation of the crystal, indicating metastability of the rapidly dehydrated protein crystal. This metastability might be analogous to that developing upon rapid withdrawal of water from crystalline hydrates due to the formation of lattice vacancies (Brown, Dollimore & Galwey, 1980).

The spontaneous shrinkage of the protein crystal is accompanied by about 25% decrease in the crystal viscosity and step-like increase of Young's modulus E shown in Fig. 2. As seen from Fig. 2, E (as well

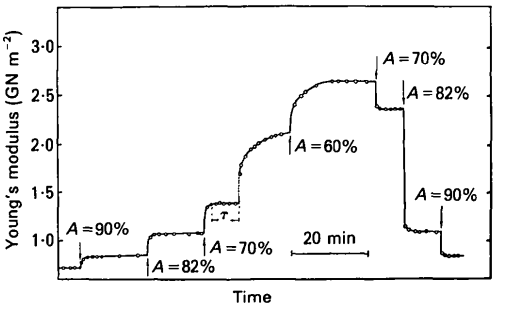

Fig. 2. Changes with time of Young's modulus induced by the humidity changes. The moments of the humidity changes are indicated by the arrows. The spontaneous transition is shown by the dotted line. For other experimental conditions see the legend to Fig. 1.

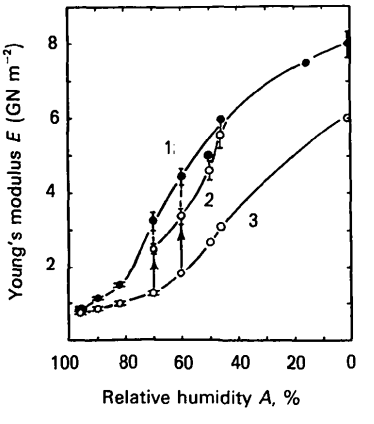

Fig. 3. Young's modulus (E) humidity dependence measured after storage for 2-3 d at each humidity point (●), the means of 4-5 experiments being shown; (○) the same measured after storage for 15-30 min, means of 20-25 experiments. For other conditions see the legends to Figs. 1 and 2.

as the length) reaches its equilibrium 1-3 min after the change of the humidity in the chamber and does not change at subsequent storage at the same humidity for 1-2 h in the region $A > 70\%$. However, at $A = 70\%$ the E, length and ϑ do undergo spontaneous step-like transition in $\tau = 2$-50 min after the equilibration of the hydration. The transient process is stochastic because the delay time τ may vary several times for the same crystal plate, other experimental conditions being equal.

The step-like transition with a duration for the step of less than 1 s is followed by the establishment in 10-20 min of some new transient equilibrium represented by curve (2) of Fig. 3. As illustrated in Fig. 2 at $A > 60\%$ both the mechanical properties and the deformation could be completely reversed upon humidification, and the transition described above could be repeated many times with the same crystal plate, but the metastability was never observed in the course of rehydration.

On storing at $A = 70\%$ for several days after the step-like transition the crystals considerably changed their mechanical properties and shrinkage reaching the values characteristic of the slow dehydration process [curves (1) of Figs. 1 and 3]. This slow relaxation is probably accompanied by accumulation of some irreversible structural changes and manifests itself in a hysteresis and residual deformation of the crystals obtained upon humidification [compare curves (1) and (2) in Fig. 1]. There is no complete recovery in diffraction patterns or lattice parameters. $d_{min} \approx 5$ Å for rehydrated crystals; 'c' and 'a, b' values are respectively 2% and 1% less than their original values. This residual shrinkage was shown to disappear only after the crystal plates had been stored for several days in a drop of their mother liquid.

It is noteworthy that the rapid decrease in humidity from $A = 82\%$ to $A = 60\%$ omitting $A = 70\%$ led to the step-like transition after $\tau = 2$-4 min, the delay time being considerably decreased due to the increase in the crystal metastability. If the transition failed to occur at $A = 60\%$ for some unknown reason (as was observed in two experiments out of 26) it was not observed on subsequent drying. A certain amount of water is likely to be necessary for the transition to occur.

It has to be admitted that both the physical nature and the kinetics of the processes occurring in a protein crystal upon drying are rather complex. Nevertheless, we believe that these processes as well as that described in the literature (Einstein & Low, 1962; Huxley & Kendrew, 1953; Perutz, 1942) can be explained based on the simple concept that the protein crystal with water activity lower than that of its mother liquid is subjected to an internal compressing pressure. This pressure tending to expand the intracrystalline liquid and to compress the protein lattice may be considered as Laplace's pressure under concave meniscuses of water appearing on the crystal surface as a result of water evaporation from open ends of intracrystalline channels (Khachaturian, 1977). Taking into account the lowering of water activity due to the presence of salt in the intracrystalline liquid we can calculate the pressure according to the Thomson (Kelvin) equation

$$P_L = -(RT/V_e) \ln (A/A_{cr}).$$

Here, A_{cr} is relative humidity over a flat surface of intracrystalline salt solution, R is the gas constant, T is the absolute temperature and V_e is the molar volume of water. With the salt concentration in the intracrystalline liquid calculated from the adsorption isotherm (Gevorkian & Morozov, 1983) A_{cr} could be found from published tables for any A. According to our calculation $P_L = 0{\cdot}035$ GN m^{-2} at $A = 70\%$. Should such a load be uniaxially applied to a material similar to the protein crystal ($E \simeq 1$ GN m^{-2}) it would be compressed by 3·5%. Good agreement between this estimate and the measured shrinkage of the protein crystal [curve (3) in Fig. 1] proves the shrinkage to be mainly the result of the action of capillary forces, the value of which may be calculated by Thomson's equation.

Since the contacts occupy about one third of the total protein surface area (Shrake & Rupley, 1973) and there are 10 contacts on each lysozyme molecule in the tetragonal crystal (Moult *et al.*, 1976), the stress applied to a contact at $A = 70\%$ will reach 0·1-0·2 GN m^{-2} according to our estimates. It is of interest that such a stress is close to the ultimate compressive stress of glassy polymers resembling the material of which the protein globule is made (Morozov & Morozova, 1981).

Both the conformational changes of protein molecules and their rearrangement in the crystal lattice might be involved in shrinkage of protein crystals. Whatever the structural basis of the shrinkage and step-like transition in tetragonal crystals, the phenomenon is likely to involve some features inherent to the deformation and failure of solids. Under low pressure applied over a relatively short time protein-crystal deformation occurs as a Hookean one, but under high pressure applied over a longer time the crystal undergoes plastic deformation or destruction.

References

BROWN, M. E., DOLLIMORE, D. & GALWEY, A. K. (1980). *Reactions in the Solid State*, edited by C. H. BAMFORD & C. I. H. TIPPES, p. 134. Amsterdam, Oxford, New York: Elsevier.

EINSTEIN, J. R. & LOW, B. W. (1962). *Acta Cryst.* **15**, 32-34.

GEVORKIAN, S. G. & MOROZOV, V. N. (1983). *Biophysics (USSR)*, **28**, 944-948.

HUXLEY, H. E. & KENDREW, J. C. (1953). *Acta Cryst.* **6**, 76-80.

KHACHATURIAN, A. G. (1977). *Zh. Eksp. Teor. Fiz.* **72**, 1149-1156.

MOROZOV, V. N. & MOROZOVA, T. YA. (1981). *Biopolymers*, **20**, 451-467.

MOULT, J., YONATH, A., TRAUB, W., SMILANSKY, A., PODJARNY, A., RABINOVICH, D. & SAYA, A. (1976). *J. Mol. Biol.* **100**, 179-195.

PERUTZ, M. F. (1942). *Nature (London)*, **149**, 491-494.
SHRAKE, A. & RUPLEY, J. A. (1973). *J. Mol. Biol.* **79**, 351-371.
STEINRAUF, L. K. (1959). *Acta Cryst.* **12**, 77-78.

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Availability of deposited atomic coordinates from the Cambridge Crystallographic Data Centre

Over the past few years an increasing number of journals have adopted the policy of depositing atomic coordinates relating to organic and metalorganic structures with the Cambridge Crystallographic Data Centre (CCDC). It appears that there has been some misunderstanding about accessing such deposited data and concern about its availability to interested scientists. This note by the CCDC is intended to clarify the situation.

Deposited data are available on request from the CCDC and a note to this effect is included in each publication which involves deposited data. The CCDC responds promptly, and free of charge, to each request either by sending a photocopy of the original deposited tables or, if the structure has already been checked and entered into the Structural Database, by sending a computer listing of the data together with other key information and a plot of the structure.

Deposited data incorporated in the Structural Database are also available by accessing tape copies of the Database distributed through National Affiliated Centres and individual laboratories in the following countries: Australia, Austria, Belgium, Brazil, Canada, CSSR, Denmark, Finland, France, Federal Republic of Germany, Hungary, India, Israel, Italy, Japan, The Netherlands, New Zealand, Norway, Saudi Arabia, South Africa, Switzerland, UK, USA and USSR. However, as indicated above, deposited data can be obtained directly from the CCDC. Deposited data are thus available worldwide independent of the distribution and currency of the Structural Database.

Book Reviews

Works intended for notice in this column should be sent direct to the Book-Review Editor (J. H. Robertson, School of Chemistry, University of Leeds, Leeds LS2 9JT, England). As far as practicable books will be reviewed in a country different from that of publication.

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Smectic liquid crystals: textures and structures. By G. W. GRAY and J. W. GOODBY. Pp. xxvi+162. Glasgow: Leonard Hill, 1984. Price £46.00.

Smectics (from the greek $\sigma\mu\eta\gamma\mu\alpha$: soap, rubber) are layered systems, like soap itself and many stearate, laurate, *etc*..., salts in water, or phospholipids in water. The mention of these chemical species is sufficient to indicate the importance of these modifications of condensed matter in applied sciences and biophysics. But, as elements of a set of materials displaying a remarkable polymorphism, these are also of intrinsic fundamental interest. This book deals with a second group of smectics, made of pure organic compounds, with elongated molecules having a rigid central aromatic part, and more or less flexible moieties of various chemical natures. Fundamental problems in chemistry, molecular and structural properties,..., are more at hand with this second group, whose study has been developed considerably in the last 15 years without showing any sign of unrest up to now, on the contrary. The same molecules are also at the origin of other liquid-crystalline mesophases, like nematic, cholesteric and 'blue' phases. The authors of this monograph, after a short introductory chapter which contains a reasonable bibliographical account of nematic, cholesteric and blue phases (also with a bibliography concerning mesophases made of plate-like molecules), turn to a detailed and systematic description of smectic polymorphism, each of the first nine chapters being devoted to one of the known smectic phases (in the alphabetic order of the terminology, *viz* *A*, *B*, ..., *I*); chapter 10 is an update of the previous chapters which, but for a few exceptions, deal with results obtained before 1982. Chapter 10 contains, in particular, a brief account of: the hexatic phase, which has proved important as a concrete example of two-dimensional melting; antiphase behaviour in the bilayered structures of nitro and cyano compounds, where the existence of a longitudinal dipole brings new interesting ordering features; and ferroelectric phases of chiral molecules, much studied today for display devices; it ends with a detailed and useful table of the structural properties of smectic phases. The whole text is completed by a beautiful series of 124 colour optical micrographs of the typical textures displayed by the various smectic modifications.

Gray and Goodby are chemists and have played an important role in the synthesis of new liquid-crystalline molecules and in the discovery of liquid-crystalline phases. With any new material of these types the standard methods of characterization used by the chemists are (1) inspection